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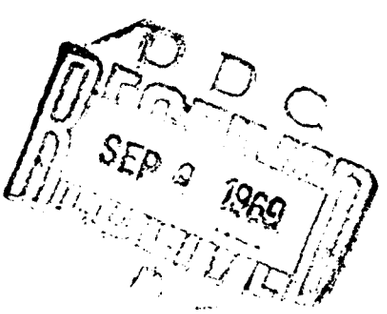


STRUCTURAL CHARACTERISTICS OF VIRAL NUCLEIC ACIDS

/Following is the translation of an article by F. L. Kiselev, Laboratory of Biochemistry, Institute of Virology imeni D. I. Ivanovskiy AMN USSR, Moscow, published in the Russian-language periodical Voprosy [illegible] (Problems of Virology) 11:5-12, 1966. It was submitted on 4 Feb 1965./

In recent years considerable successes have been achieved in the study of the chemistry of viral nucleic acids. There was special interest in clearing up the conjectural peculiarities of the structure of the hereditary substance (DNA or RNA) of the virus as an intracellular parasite, as a result of which the nucleic acid of the virus, when it penetrates into the cell, suppresses the function of host genome and causes a shift of cell metabolism to the side of individual components of virus particles.

The minimum volume of viral suspension necessary for a physicochemical study of nucleic acids of viruses comprises several tens of liters with a particle content of 10^9 in 1 ml. With the help of methods of differential centrifuging, ion-exchange chromatography, treatment with appropriate enzymes, and a number of other methods it is possible to obtain highly purified suspensions of viruses which are suitable for the isolation of nucleic acids.

At the same time viral nucleic acids have a number of advantages over tissue. First of all in viruses there is no diverse nature of nucleases, which makes it possible to obtain preparations of nucleic acid in a highly native state. Moreover, later works , dealing with the organization of chromosomes in animal tissues /1/, testify that in the make-up of a chromosome there is only one molecule of DNA, the length of which may reach several millimeters and molecular weight - 10's of billions. It is natural that it is hardly possible to obtain preparations of native DNA with such dimensions. The molecular weight of viral nucleic acids does not exceed 120--150 million, and if a number of precautions are observed, it is possible to obtain a molecule of viral nucleic acid with the minimum amount of damages.

The first successes in the study of the chemistry of viral nucleic acids were connected with bacteriophages. In them many qualitative deviations were detected in the composition of nucleic acids. First of all the DNA of T-even phages contain other carbohydrates besides deoxyribose: glucose and gentiobiose disaccharide, and 5-oxymethylcytosine completely replaces cytosine /2-8/. At present

15 phages are known, the DNA of which includes 5-oxymethylcytosine, [9-10]. In the make-up of DNA of Bac. subtilis phages 5-oxymethyluracil has been detected [11]. As an additional carbohydrate component the SP 8 wild strain of phage contains d-glucose, and in its thermosensitive mutant SP 8 T_s, in place of d-glucose - d-monnose [12], whereas, as the author suggests, these supplementary components are confined to only one thread of DNA. Somewhat unexpected was the discovery of uracil, an indispensable component of RNA, in the DNA of transducing phage of B. subtilis PBS 2 [13].

Besides the qualitative deviations, which most probably are connected with the process of inversion of metabolism in the cell, the nucleic acids of viruses display a number of other peculiarities.

Szybalski [14], having established the unusually high thermostability of the chickenpox virus, expressed the assumption that the threads of the double-helical nucleic acid of this virus are bound by covalent bonds. Polynucleotide chains of DNA of SP6 and SP8 phages, obtained as a result of denaturing, differ in their specific gravity, which is connected with the significant predominance in one of the threads of either purine or pyrimidine bases [15-17]. Moreover, Strauss demonstrated that the RNA of MS 2 phage is not precipitated by 1 M NaCl, while ordinary single-helix RNA is precipitated in the salt of this concentration [18].

We will not examine those viral nucleic acids which represent "ordinary" DNA or RNA, such as the T-even phage DNA or polio virus RNA for example, but will switch to an analysis of those viral nucleic acids, the structure of which differs noticeably from normal.

In 1959 Sinsheimer and Tessman [19-22], while studying the process of inactivation of phages T_4 and ΨX 174 which were tagged with P^{32}, detected that the latter is inactivated 10 times more rapidly than T_4. Mutations under the influence of nitrous acid in the ΨX174 phage developed only in pure clones, and in T_4 phage - in mixed clones. Consequently the ΨX174 phage should have possessed one copy of genetic material, i.e., its DNA should be single-helix. The fact that this is actually DNA, and not single-chain RNA, was confirmed by the fact that purified preparations of this DNA gave a positive reaction to deoxypeptose and were hydrolyzed by DNAse and not RNAse. On the basis of what was said above, Sinsheimer and Tessman proposed that the DNA of ΨX174 phage is single-helix. Subsequently this proposal was confirmed completely. The nucleotide composition of this DNA did not satisfy the rule of Chargaff; this DNA reacted with formalin, which testified to the presence in it of free amino groups, while in ordinary double-helix DNA the amino groups are blocked. Absorption in ultraviolet light was increased gradually in a wide range of temperatures (from 20 to 60°), while in ordinary double-strand DNA this transition takes place in a narrow temperature range. The floating density of DNA of the ΨX 174 phage, determined by the method

of equilibrium centrifuging in a gradient of density, turned out to be equal to 1.72 g/cm^3, while the density of ordinary DNA comprised 1.70 g/cm^3. Besides this, absorption of the solution of DNA in ultraviolet light depended on ionic strength and pH of the solution. Thus in contrast to double-helix, single-helix is able to change its form depending on various conditions of the medium. At present 6 bacteriophages are already known which contain single-chain DNA [23].

Subsequent investigation of the DNA of ΨX174 phage revealed yet another peculiarity. Fiers and Sinsheimer [24] studied the effect of exonuclease on the DNA of ΨX174 phage. It turned out that neither phosphodiesterase of E. coli, nor phosphodiesterase of viper venom, nor phosphatase hydrolyzed DNA (in the absence of the above-stated enzymes of DNAse). Following treatment with DNAse the preparation acquired a sensitivity to phosphodiesterase, but such hydrolysis never reached the end. It follows from this that the DNA of ΨX174 phage does not contain 3'- and 5'-hydroxyl, and also terminal phosphate groups, and, in addition to this, within the DNA molecule there is some section which is resistant to phosphodiesterases. In preparations of DNA from ΨX174 phage two components were revealed with sedimentation constants of 13,1S (S_1-component) and 12,1S (S_2-component). Infection is connected only with the S_1-component. Under the influence of DNAse the S_1-component is converted into the S_2-component, which is sensitive to phosphodiesterases. On the basis of the results obtained Fiers and Sinsheimer came to the conclusion that native DNA of ΨX174 phage represents a cyclic closed structure; its ends are joined by some bond, the nature of which is still not clear at present. This makes comprehensible the mechanism of action of nucleases on this DNA; DNAse, which possesses a non-specific action, disrupts the molecule of DNA and only after this the corresponding phosphodiesterases begin hydrolysis with the ends of the molecule and move forward along it until they come across a section with a more stable bond.

Thus the nucleic acid of ΨX174 bacteriophage represents single-helix cyclic DNA.

No less interesting forms of DNA are the double-helix forms of DNA with cyclic structures. They were discovered during investigation of the viruses of polyoma [25] and Shoup papilloma [26, 27]. Dulbecco [25], while studying the DNA of polyoma virus, detected two forms of nucleic acid: an F-form which settled rapidly during ultracentrifuging and the slowly settling S-form.

Both these forms are typical double-helix structures with the characteristic intensity of hyperchromic effect, buoyant density, and high temperature of melting (around 100°). Dulbecco considers that the S-form represents a linear polymer, and the F-form - a cyclic structure. The author suggests that the linear structure closes into cyclic with the help of a special "lock," which is

confirmed in experiments with DNAse. The F- and S-forms are infectious, but if an S-form is obtained from an F-form with the help of DNAse, which possesses a non-specific action, there is a loss of infectious capacity, i.e., a fracture of the molecule, not through the "lock" but in some other place. Vinograd and associates [29] introduced several supplementary corrections in the structure of DNA of the polyoma virus. They demonstrated that after treatment of the F-component, sedimentation constant 20S, with DNAse in actuality the S-component with a sedimentation constant of 16S is formed, but this component is not linear. Electron microscopy of both preparations revealed only circular structures. The authors propose that the F-component of the polyoma virus is a twisted coil made up of double-strand threads. Such a conformational change naturally leads to the formation of a comparatively densely packed structure with higher sedimentation characteristics. The S-component is an ordinary circular double-strand molecule without any sharp bends, naturally having a lesser value of sedimentation constant due to a "looser" structure.

The two-component state of DNA of the Shoup papilloma virus was observed earlier by Watson [26], however, the conclusion concerning the cyclic nature of DNA was not followed then. According to the findings of Watson the DNA which is isolated from this virus has sedimentation constants of 21S and 28S. Watson's findings were confirmed by Crawford [27]. The 28S component turned out to be cyclic, and the 21S - linear (in the author's opinion). The circular nature of DNA from T_2 phage was also demonstrated [29].

Up until now we have examined viruses which have only one type of nucleic acid: either double-helix or single-helix DNA. There is considerable interest in the report by Pfau [30, 31] concerning the simultaneous presence in the smallpox vaccine virus of double- and single-helix DNA. Initially Pfau reported that with the help of phenol it was possible to isolate a preparation of DNA which made up 15% of the total amount of viral DNA and possessed properties which were characteristic for single-helix DNA. Then with the help of 2-mercaptoethanol and pronase (proteolytic enzyme from cells of Streptomyces griseus) he was able to achieve the complete liberation of DNA from the viral particle. By gradient centrifuging of such a preparation of DNA in CsCl it was divided into two zones. One zone, which contained 12% of all the DNA, had a specific gravity of 1.724 g/cm^3 and represented single-helix DNA, which in the first experiments the author extracted with phenol. The second zone, which made up 85-88% of the material, had a specific gravity of 1.706 g/cm^3 and represented typical double-helix DNA. Upon heating the first type of DNA showed a gradual increase of absorption in ultraviolet light, and the second type possessed a specific melting point of 84°. The author subjected the initial preparation of smallpox vaccine virus to fractionation with the help of centrifuging in a density gradient of calcium tartrate. It turned out that the initial preparation contained two types of viral particles. In the heavier particles only double-helix DNA was found, and in the lighter ones - double-helix and single-helix; the latter comprised 40%.

Electron microscopic investigation also confirmed the presence of two types of viral particles. One of them turned out to be permeable for phosphomolybdic acid, and the other was not. The main mass of viral particles was non-permeable for phosphomolybdic acid. The DNA of these particles is double-helix and is not extracted with phenol, and it comes forth into the medium only after preliminary treatment of the viral suspension with mercaptoethanol.

These findings by Pfau give rise to a number of questions. Is one of the types of viral particles the precursor of the other? If yes, then which? Or are both forms of particles similarly capable of reproduction and exist independent of each other and simultaneously? Yet the smallpox vaccine virus remains a puzzle for virologists.

The greatest interest, particularly for biochemists, was caused by the detection of double-helix RNA in reoviruses [32-34] and in the virus of wound galls of plants [34]. Reoviruses possess a number of peculiarities which distinguish them from ordinary RNA-containing viruses. First of all their rate of multiplication is lower; secondly, staining of particles with acridine orange causes a yellow-green fluorescence, which is characteristic for DNA; thirdly, reproduction of the reovirus is inhibited by actinomycin D - an antibiotic which suppresses the synthesis of RNA on matrix DNA. The reproduction of ordinary RNA-containing viruses is not inhibited by this antibiotic. Fourth, infection of sensitive cells by reoviruses suppressed the synthesis of host DNA, while the synthesis of RNA and protein continues.

Based on these findings, Gomatos [32] expressed the assumption that reovirus RNA is double-helix. Subsequent experimental investigations confirmed this assumption. First of all the nucleotide composition of this RNA satisfies the rule of Chargaff for double-helix DNA, i.e., the ratio of the number of purines and pyrimidines was equal to 1. The RNA of reoviruses is resistant to pancreatic RNAse. The absorption spectra in ultraviolet light does not depend on value of ionic strength, pH, and temperature of the medium. This RNA does not react with formaldehyde and has a strictly specific melting point - 100°. It is necessary to note that DNA with the same percentage composition of guanine-cytosine (40%) melts at 87°. This testifies to the fact that the double polyribonucleotide chain of this RNA is extremely stable. Presumably such a stability is explained by the formation of a hydrogen bond between the second hydroxyl of ribose and the phosphate inside one chain [38].

Viral RNA from wound galls of plants possesses properties which are similar with the RNA of reoviruses.

Double-helix RNA displayed still another interesting property - an unusually high affinity for degradation. During electron microscopic investigations of these RNA's, conducted by Kleinschmidt and associates [35] and Gomatos and Stocknius [36], it was not possible

to detect fragments of DNA with a molecular weight greater than 4 million. The main mass of RNA molecules was represented by fragments with a molecular weight of 1,000,000, and the degradation of molecules was found in dependence on the hydrodynamic influences used for the isolation of RNA. In a comparison of the findings of electron microscopic investigation with sedimentation characteristics the authors /36/ come to the conclusion that double-helix RNA in a solution has a more compact configuration than double-helix DNA. Langridge and Gomatos /37/, and also Tomita and Rich /38/, carried out a detailed X-ray investigation of these RNA's. For RNA of reoviruses the pitch of the helix turned out to be equal to 30.5 Å, number of fragments per turn 10, distance between bases 3.05 Å, angle of tilt to the axis of the helix 10-15°, angular rotation of base 36°. For DNA in the A-configuration (at 72% relative humidity) these values equal correspondingly 28.1 Å, 11, 2.5 Å, 20°, and 33°. For DNA in the B-configuration (92% relative humidity) they are 34.6 Å, 10, 3.4 Å, 0°, and 36° respectively.

From the point of view of studying the molecular structure of nucleic acids there is considerable interest in the group of myxoviruses, the most complexly organized group among the RNA-containing viruses.

As a model we used the parainfluenza Sendai virus. The selection of this virus is explained mainly by the possibility of obtaining high initial titers of infectivity (of the order 10^9-10^{10}).

Purification and concentration of virus was carried out by a method which we described earlier /39/. The RNA obtained from suspensions which were purified in such a manner produced a spectrum of absorption which is characteristic for nucleic acids, with a maximum at 258 nm. In media of various ionic strength the value of absorption of Sendai virus RNA does not depend on ionic strength. We recall that absorption of DNA also does not display a dependence on ionic strength, which is connected with the rigid double-helix structure.

An investigation of the change in RNA absorption depending on temperature (melting curve) showed that up to approximately 85-90° absorption does not change, but then it increases sharply, having a maximum at 102-104°. Melting temperature of DNA is fixed and is equal to approximately 85° in 0.1 M NaCl.

The greatest hyperchromic effect for RNA of Sendai virus, obtained by means of heating a solution with 2% formalin at 104° for 20 minutes, equals 57%, for reovirus RNA it is more than 50%, for phage DNA - 50%, and for single-helix RNA it does not exceed 20%. Heating of Sendai virus RNA up to $>90°$ with a subsequent rapid cooling leads to the complete restoration of absorption, while for DNA such an effect is not observed /40/.

A study of the kinetics of the reaction of Sendai virus RNA with RNase revealed its resistance to the action of the enzyme. For RNA of the tobacco mosaic virus the hyperchromic effect during incubation with RNase in the same concentration reached 20% in 0.1 M NaCl, and for Sendai virus RNA it was absent. Incubation of Sendai virus RNA with 2% formalin showed the resistance of that RNA to the action of formalin. In these tests the 4-6% of hyperchromism is most probably connected with the partial degradation of material in the process of isolation. At the present time this problem is being cleared up.

The data cited above testify that the RNA of the Sendai virus has a structure which is similar to that of DNA, i.e., double-helix. At present we do not have available the necessary data to judge whether this helix consists of two independent polynucleotide chains or one which is twisted around itself, as this has been established for T-RNA.

As is known, the majority of ribonucleic acids investigated have a molecular weight no greater than 2,000,000. Exceptions are certain viral RNA's (reovirus, the virus of wound gall of plants, Rous sarcoma virus) which have a molecular weight of 10,000,000. Proof of the double-helix nature of RNA of the first two viruses naturally presumed a similar structure also for the RNA of the Rous sarcoma virus. However, Robinson and associates [41] refuted these proposals. The sedimentation constant for RNA of this virus turned out to be equal to 62S, i.e., doubled the $S_{20, W}$ for RNA of the tobacco mosaic virus. The nucleotide composition of sarcoma virus RNA is typical for single-helix RNA. Its thermal denaturing did not cause displacement of the zone of RNA in the density gradient CsCl. Molecular weight comprised $9.6 \cdot 10^6$. Until now single-helix RNA with such a high molecular weight has not been detected. It is necessary to note that the RNA of the Rous sarcoma virus which was studied did not represent a pure preparation, since the Rous virus multiplies in cells only in the presence of a virus-"assistant" - one of the viruses causing fowl leucoses, and the initial preparation of RNA consisted of a mixture of both viruses. At present it is difficult to say if the high molecular weight of RNA is connected with this peculiarity of multiplication of RNA or if some other properties determine this anomaly of RNA.

There is no doubt that the subsequent detailed study of the structure of nucleic acids of viruses will make it possible to reveal many new findings in this area.

In the process of development of a virus two stages are clearly distinguished: the virus outside of the cell and the virus in the cell. It could hardly be assumed that it was possible to reveal any differences in the structure of nucleic acids of the vegetative and quiescent virus.

These differences were first revealed in the multiplication of ΨX174 phage, the DNA of which, as was stated, represents the single-helix cyclic form in the quiescent virus. Sinsheimer isolated DNA from ordinary phage and phage DNA from an infected cell and subjected both preparations to equilibrium centrifuging in a CsCl density gradient. It turned out that the value of floating density of DNA from quiescent phage was greater than DNA of the vegetative virus. Immediately after infection "heavy" phage DNA was converted into "light," and this "light" form, included in the composition of newly formed particles, turned out to be "heavy." Both forms were infectious. The "light" intracellular DNA received the name of the replication form.

Hayashi and associates [42] isolated and purified this replication form by means of repeated chromatography on columns with methylated albumin. This form displayed a melting curve which was typical for double-helix DNA. During heating the same hyperchromic effect was observed as in the case of using ordinary DNA, and the "heavy" phage DNA converted into the "light." Floating density of the replication form turned out to be equal to 1.707 g/cm^3, which is characteristic for the double-helix structure. Sinsheimer confirmed that this was in actuality double-helix DNA, and not a DNA - RNA hybrid, by the fact that this form is hydrolyzed by DNAse and that depolymerase does not act on hybrid forms. Then it was shown that the replication form possesses a cyclic structure; this was confirmed by direct electron-microscopic investigation [43, 44].

While studying the multiplication of T_2 phage, Fraenkel [45] detected that viral DNA, isolated from infected cells, differs from ordinary DNA from mature virus particles. If one were to judge from the results of centrifuging in a density gradient, then both forms have a double-helix structure. But the replication form displays a greater affinity for an exchanger; in a density gradient of saccharose it is precipitated 1.21-1.35 times more rapidly. The author does not make any conclusions regarding these peculiarities.

Interesting data were obtained by Kozinski during a study of replication DNA of T_4 phage [46]. In particular he demonstrated that during multiplication phage DNA forms a specific complex with the protein which existed in the cell prior to infection. In 5-6 minutes after infection the DNA in this complex displays many new properties: it is circular, has somewhat greater dimensions, and possibly is partially denatured.

Finally the replication forms of RNA-containing viruses. Montagnier and Sanders [47], while studying the multiplication of the virus of encephalomyocarditis in a culture of Krebs-2 cells, demonstrated that the main mass of viral RNA has a sedimentation constant of 37S and the structure of a single polynucleotide chain. However, there was a small fraction of RNA, the amount of which reached a maximum by 6 hours after infection with a sedimentation

content of 60%. Treatment of the whole preparation of RNA with RNAse led to the complete disappearance of the RNA fraction with the sedimentation constant of 57S, while the 20S RNA turned out to be resistant to the action of the enzyme. With a lowering of ionic strength or an increase of temperature the resistance to RNAse was lowered. Molecular weight turned out to be equal to $6.7 \cdot 10^6$, i.e., triple the molecular weight of RNA from virus particles. Melting temperature in 0.15 M NaCl was 95°.

Similar properties were possessed by the replication form of RNA from the poliomyelitis virus [48, 49].

The most accurate findings concerning the properties and methods of formation of replication forms of nucleic acids for RNA-containing viruses were obtained by Weissman and associates [50-52]. During a study of the multiplication of the RNA-containing MS2 phage, which was labeled with P^{32}, they demonstrated that in 6 minutes after infection an RNA fraction emerges which is resistant to RNAse: the content of this fraction reaches a maximum by the 15th minute, when 15% of RNA - P^{32} becomes resistant to the action of RNAse, then the amount of this fraction decreases. The melting temperature of this replication form is 102° in 0.15 M NaCl and 0.015 M citrate. After heating up to the specified temperature and a subsequent rapid cooling a sensitivity to RNAse appeared. The floating density of the replication form in Cs_2SO_4 is 0.02 units lower than for RNA of the MS2 phage. After thermal denaturing with annealing in the presence of phage RNA a product was formed which possessed the properties of the replication form.

In addition to experiments in vivo, the authors made attempts to obtain a similar replication form in vitro. Preliminarily it is necessary to note that from cells which were infected with RNA-containing viruses it was possible to isolate the enzyme RNA-synthetase, which carries out the synthesis of RNA on matrix RNA. The emergence of such an enzyme was demonstrated in the cells of mammals [48, 49] and bacteria [52, 53].

It was revealed that RNA-synthetase, which is present only in infected cells, is obligatorily associated with the replication form of RNA and in systems in vitro carries out the synthesis of both double-thread and single-thread RNA from nucleotide triphosphates without the addition of an exogenous matrix [54]. Weissman [55] proposes that in vivo this process, i.e., the above-mentioned synthesis, is secondary, and the conversion of parent RNA into the replication form is primary. The realization of this primary phase takes place with the help of an enzyme, the nature of which has still not been clarified.

To clear up whether or not both chains of the replication form are synthesized de novo or if one of them is parent, Weissman [52]

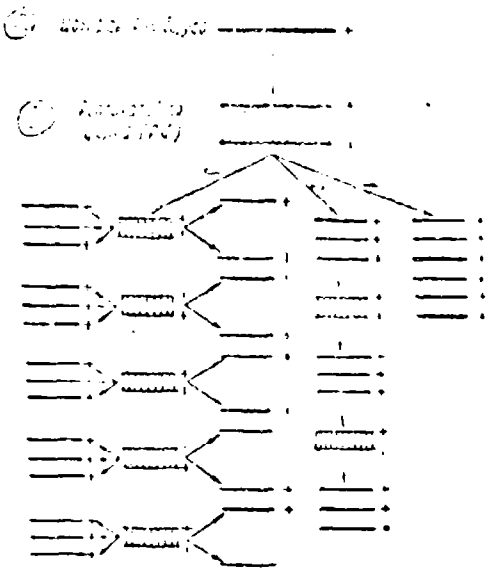
studied the distribution of P^{32} in the molecule. It turned out that radioactivity is found only in the (parent) polarized strand, i.e., then the second is synthesized anew.

Two polymorphic forms of RNA have been isolated in plant viruses [6-8] and arboviruses [5]. Synthesis of the replication form of RNA of the poliomyelitis virus has been carried out in vitro [9].

The study of the properties of the replication forms made it possible to clear up many peculiarities in the multiplication of RNA-containing viruses; simultaneously a number of new problems arose, the answers to which have still not been obtained. These problems can basically be reduced to the following. In a quiescent state the RNA inside a virus represents a single-helix structure (an exception are viruses with double-helix RNA). In the period of multiplication this single-helix RNA becomes double-helix (with the establishment of the replication forms of RNA of plant viruses and the Semliki Forest virus this can hardly cause any doubt). Here the second polynucleotide chain, as was pointed out by Weissmann, is synthesized anew. The position that the formation of a double helix cannot take place by means of a polynucleotide chain twisting around itself is confirmed by the fact that the molecular weight of the replication form of RNA of the encephalomyocarditis virus turned out to equal $6.7 \cdot 10^6$, and in the case of twisting around itself it should not change, i.e., exceed $2 \cdot 10^6$. The structure of the replication form is similar to the structure of double-helix reovirus RNA [11].

The nucleic acid of the parent particle has a strictly specific sequence of nucleotides [(+) sequence]. In this case the newly synthesized second polynucleotide chain of the replication form should naturally have a sequence which complements it, i.e., an antisequence [(-) sequence]. For example, if the (+) chain has the sequence AAGUA, then the (-) chain will have the sequence UUCAC. It would seem that further synthesis of single-helix chains could take place on each of the two helixes of the replication form. But for the system of synthesis of RNA on a matrix of DNA it has been shown by the works of Hayman and associates [10], Spiegelman and associates [12], and Goldthwait and associates that synthesis bears an asymmetrical nature, i.e., takes place only on one of the two chains of DNA, and the synthesized RNA is identical in the sequence of nucleotides to one of the chains of DNA and correspondingly complimentary to the other. The asymmetrical nature of synthesis has also been proven for the system replication RNA - single-strand RNA [5]. For antisequence, i.e., for (-) chain, there is also the possibility of synthesis of viral RNA, i.e., the (+) chain, since only in this case will the sequence of nucleotides in it correspond to viral. On the (+) chain once again the (-) chain may be synthesized, etc. The possible mechanisms of this process are presented schematically in Figure 1.

Fig. 1



Possible means of replication of nucleic acids in RNA-containing viruses.
(a) Initial RNA of virus; (b) Replication form (RF).

The first possibility is that from one molecule of the initial viral RNA one molecule of the replication form is formed, and on its (-) chain a great number of molecules of viral RNA is synthesized, i.e., a (+) chain. However, this is not very probable if it is taken into consideration that for one molecule of the replication form there are 10⁴ molecules of viral RNA, since 10% of the viral RNA in the cell is resistant to RNase. Another possibility is that after the formation of one molecule of the replication form on it there is the synthesis, in the manner of DNA, of a strictly specific number of such replication forms. Then each of the replication forms either dissociates into (+) and (-) chains, which is hardly possible since all the (-) chains cannot enter into the composition of the newly formed viral particle, or only (+) chains are synthesized on these replication forms. The third path also begins with the formation of a unique molecule of the replication form, on which only (+) chains are synthesized. Since the capacity of the replication form for the synthesis of new (+) chains is lost (possible due to "aging" of the molecule), one of the newly formed (+) chains synthesizes a (-) chain, i.e., a new replication form is formed.

The most complete answer to these questions is given in the review by Ochoa and Mehler [69], where the authors propose the following system of replication of RNA-containing viruses. The initial parent RNA carries out two basic functions: 1) the function of information RNA, programming the synthesis of capsid proteins, and 2) the

function of induction of the formation of one, and possibly two, RNA-synthesizing enzymes. The parent RNA is converted into a double-helix replication form, on which the synthesis of daughter molecules of RNA takes place in an asymmetric semiconservative pattern, i.e., there is initially the synthesis of (+) chains on the (-) chain of the replication form (asymmetric nature of synthesis). Newly formed (+) chains dislodge the parent molecule from the replication form (semiconservative nature). The question of how (-) chains are synthesized and utilized remains unanswered.

An analysis of the above-listed peculiarities in the structure of nucleic acids of both quiescent and vegetative viruses at the given stage does not make it possible to draw specific conclusions concerning the significance of these peculiarities in individual stages of virus multiplication. Further investigations are necessary for clearing up the significance of anomalies in the structure of viral nucleic acids in the process of interaction of the virus with the cell

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